horticulturae

MDPI

Article

Impacts of Epihomobrassinolide and Thiamethoxam·Flutolanil·Azoxystrobin on the Continuous Cropping Stress of *Pinellia ternata*

Bing Tian [1,2,†], Chenglin Tang [1,3,†], Jiaqi Liu [1], Qiuping Wang [4,5], Wenhao Feng [3], Yue Su [4], Cheng Zhang [1,*] and Yang Lei [4,*]

[1] School of Public Health, Guizhou Medical University, Guiyang 550025, China; tianbing565@163.com (B.T.); cl_tang520@163.com (C.T.); gmcjiaqiliu@126.com (J.L.)
[2] Guizhou Agricultural Ecology and Resource Protection Station, Agriculture and Rural Affairs Department of Guizhou Province, Guiyang 550001, China
[3] Guizhou Crop Technology Extension Station, Agriculture and Rural Affairs Department of Guizhou Province, Guiyang 550001, China; gzfwh126@126.com
[4] Department of Food and Medicine, Guizhou Vocational College of Agriculture, Qingzhen 551400, China; qpwang518@aliyun.com (Q.W.); suyue09136@163.com (Y.S.)
[5] Institute of Crop Protection, College of Agriculture, Guizhou University, Guiyang 550025, China
* Correspondence: chengz76@gmc.edu.cn (C.Z.); gznzyylei@126.com (Y.L.)
† These authors contributed equally to this work.

Abstract: Continuous cropping (CC) stress severely limits the growth and industrial development of *Pinellia ternata*. Epihomobrassinolide (EBR) is a natural product that widely participates in many the physiological activities of many plants. Thiamethoxam·flutolanil·azoxystrobin (TFA) has been registered as a seed coating agent in crop production. In this work, the effects of seeds soaked with EBR, seeds coated with TFA, and their co-application on the plant growth, electrophysiological information (as physiological activities related to plant electrical signals), leaf photosynthesis, plant resistance, bulb quality, and yield of CC *P. ternata* were evaluated. The aim of this work is to excogitate a practicable agronomic measure for ameliorating the growth of CC *P. ternata*. The results show that soaking the seeds with EBR or coating the seeds with TFA could effectively enhance the plant height, leaf area, and stem diameter of CC *P. ternata*, promote its emergence seedling ratio, and decrease its inverted seedling ratio, and their associated application was found to be more efficient. Additionally, their associated application effectively enhanced the intrinsic capacitance (IC), intracellular water metabolism, nutrient transport, and metabolic activity and decreased the intrinsic resistance (IR), impedance (IZ), capacitive reactance (IXc), and inductive reactance (IXL). Meanwhile, their associated application could reliably enhance the photosynthetic capacity and stress resistance, and effectively improve the bulb quality and yield. This study emphasizes that the associated application of seeds soaked with a 0.004% aqueous EBR solution diluted 1000 times and seeds coated with a 24% TFA flowable concentrate at 1.6 mL kg^{-1} seed can be used as a novel and practicable technology for alleviating the CC stress of *P. ternata* and ameliorating its growth, electrophysiological information, resistance, quality, and yield.

Keywords: seed coating agent; electrophysiological information; growth and resistance; quality and yield

Citation: Tian, B.; Tang, C.; Liu, J.; Wang, Q.; Feng, W.; Su, Y.; Zhang, C.; Lei, Y. Impacts of Epihomobrassinolide and Thiamethoxam·Flutolanil·Azoxystrobin on the Continuous Cropping Stress of *Pinellia ternata*. *Horticulturae* **2024**, *10*, 696. https://doi.org/10.3390/horticulturae10070696

Academic Editors: Sergio Ruffo Roberto and Julė Jankauskienė

Received: 25 May 2024
Revised: 26 June 2024
Accepted: 27 June 2024
Published: 1 July 2024

1. Introduction

Pinellia ternata (Thunb.) Breit., an ornamental and medical Chinese herb rich in polysaccharides, alkaloids, nucleosides, minerals, flavonoids, and phenolics, has high medicinal and economic value [1–3]. Its dried bulb has multiple pharmacological functions, such as resolving phlegm, stopping vomiting, eliminating stagnation, reducing swelling, curing coronary diseases, protecting the liver, decreasing blood fat, and functioning as

a antineoplastic agent, etc. [4–7]. It is mainly produced in China, serving as an efficient means of revitalizing rural areas and alleviating poverty, and is generally planted in the provinces of Gansu and Guizhou, China, which comprise planting areas of over 1333 and 4000 hm^2, respectively [8].

However, continuous cropping (CC) stress severely limits the growth and industrial development of *P. ternata* and constantly generates economic losses of 40–60% [9,10]. The process of CC means that the same crop has been planted on the same land for more than 2 years, and this process has many obstacles or problems, including inhibiting growth, deteriorating quality, aggravating diseases and pests, and decreasing yield, as well as acidulating soils, sealing soil nutrients, and unbalancing the soil microbial structure [11–14]. Therefore, it is of realistic significance to develop a variety of practicable cultivation techniques for controlling the obstacles or problems of the CC of *P. ternata*.

Generally, intercropping, disinfection, and rotation are applied to alleviate the CC obstacles of *P. ternata* [13–15]. For instance, Hang et al. [15] indicated that soybean intercropping effectively enhanced *P. ternata* yield and pepper intercropping evidently improved its guanosine and succinic acid contents. He et al. [13] found that rotation remediated the worsening microbial community in soils of CC *P. ternata*. Nevertheless, these measures had the same disadvantages, such as poor operability, low efficiency, and complicated processes, which severely restricted their application and promotion. Furthermore, fertilizers and pesticides are often subjected to increased application by medicinal herb farmers to maintain the *P. ternata* yield, which can easily lead to issues regarding medicinal material safety and environmental pollution. Due to the severity of the CC obstacles, more practicable measures must be exploited to promote the plant growth, medicinal quality, and bulb yield of CC *P. ternata*.

Epihomobrassinolide (EBR), a novel steroidal hormone or biostimulant, widely participates in many plants' physiological activities, including cell division, cell elongation, seed germination, root development, flowering, senescence, and stress responses [16–20]. For example, Li et al. [21] found that brassinolide could regulate seed germination and root development via BES1-mediated transcription. Kolomeichuk et al. [22] reported that EBR could enhance the photosynthetic pigments, electron transport, and photosystem II maximum of *Solanum tuberosum* L. for alleviating salt stress. Moreover, it was found that EBR could also increase the proline content of *Elymus nutans* and reduce its MDA and ROS accumulation to enhance its cold tolerance [23]. Nevertheless, so far, there are no studies available in the literature about EBR improving the growth of CC *P. ternata* and alleviating its CC stress through seed soaking.

Thiamethoxam, a second-generation class III neonicotinoid (insecticide), has been made available and registered for various crops such as cereals, vegetables, and fruits. It is widely applied in seed coatings, foliar spraying, and soil drenching [24–26]. Flutolanil, a benzoylanilide fungicide, has excellent antifungal activity against Rhizoctonia pathogens, which often lead to crop root diseases [26,27]. Azoxystrobin, a broad-spectrum strobilurin fungicide, has been widely applied in the control of blast, powdery mildew, scab, and rust diseases in various cereals, vegetables, and fruits [28–30]. In China, thiamethoxam·flutolanil·azoxystrobin (TFA), a compound formulation, was registered as a seed coating agent for wheat and potato production. In the same way, up to now, there is no documentation of whether TFA enhances CC *P. ternata* growth through seed coating.

Overall, whether seeds soaked via EBR, seed coated with TFA, and their co-application can enhance CC *P. ternata* growth and alleviate its CC obstacles are also worth further exploration. In this work, the effects of seeds soaked with EBR, seeds coated with TFA, and their co-application on the plant growth, electrophysiological information, leaf photosynthesis, and plant resistance of CC *P. ternata* were first investigated. Additionally, their effects on the bulb quality and yield of CC *P. ternata* were also evaluated. The aim of this work is to excogitate a practicable agronomic measure for ameliorating the growth of CC *P. ternata*.

2. Materials and Methods

2.1. P. ternata Orchard

The field experimental orchard of *P. ternata* was located in Baiyi Training Base of Guizhou Vocational College of Agriculture in Wudang county, China (26°83′02″ N, 106°94′47″ E); this land has been planted with *P. ternata* for two consecutive years (i.e., continuous cropping land). Additionally, other land adjacent to the aforementioned land that has never been planted with *P. ternata* was also used for control experiments (i.e., no continuous cropping land). In this work, a seed soaking in EBR + seed coating with TFA experiment was carried out in the third year. Moreover, the sunshine, temperature, rainfall, altitude, and frostless season in the *P. ternata* orchard were about 1134.12 h, 13.5 °C, 1184 mm, 1330 m, and 288 d, respectively. In addition, its soil fertility is displayed in Table 1.

Table 1. The soil fertility of the *P. ternata* orchard.

Fertility	Content (g kg^{-1})	Fertility	Content (mg kg^{-1})
Organic matter	15.02	Available iron	6.51
Total nitrogen	1.45	Exchangeable magnesium	305.18
Total phosphorus	1.71	Available zinc	0.95
Total potassium	1.15	Available manganese	16.33
pH	6.35	Available copper	1.06
Exchangeable calcium	19.41 cmol kg^{-1}	Available boron	0.28

2.2. Hormone, Seed Pesticides, and Seed

The 0.004% 28-epihomobrassinolide (EBR) aqueous solution (AS) was produced by Yunnan Yunda Technology Agrochemical Co., Ltd. (Kunming, Yunnan, China). The 24% thiamethoxam·flutolanil·azoxystrobin (TFA) flowable concentrate for seed treatment (FC) was obtained from Jiangyin Suli Chemical Co., Ltd. (Jiangsu, Jiangying, China). *P. ternata* seeds (0.8~1.2 cm of diameter) were purchased from Hezhang Mountain Efficient Agricultural Technology Co., Ltd. (Bijie, Guizhou, China), its variety name is 'Hemayu 1', and the seed production year was 2022.

2.3. Seed Soaking and Coating Experiments

Riding planting of *P. ternata* took place in an orchard, the experimental plot area was 4.0 m^2 (1.0 m width, 4.0 m length, and 0.2 m in between), and the experimental plots were delineated through a completely randomized method. Seven treatments for the CC group were designed as follows:

(1) EBR1-TFA1—*P. ternata* seeds soaked with 0.004% EBR AS in a 1000-times dilution liquid + *P. ternata* seed coated with 24% TFA FC at 1.6 mL kg^{-1} seed;
(2) EBR1-TFA2—*P. ternata* seeds soaked with 0.004% EBR AS in a 1000-times dilution liquid + *P. ternata* seeds coated with 24% TFA FC at 2.0 mL kg^{-1} seed;
(3) EBR1—*P. ternata* seeds soaked with 0.004% EBR AS in a 1000-times dilution liquid;
(4) EBR2—*P. ternata* seeds soaked with 0.004% EBR AS in a 1500-times dilution liquid;
(5) TFA1—*P. ternata* seeds coated with 24% TFA FC at 1.6 mL kg^{-1} seed;
(6) TFA2—*P. ternata* seeds coated with 24% TFA FC at 2.0 mL kg^{-1} seed;
(7) C1—*P. ternata* seed soaked with clear water (intragroup control).

Moreover, one treatment for the no CC group was designed as follows:

(8) C2—no continuous cropping of *P. ternata* seeds soaked in clear water (intergroup control).

Seed soaking: Distilled water was applied for dissolving and diluting the EBR, and the *P. ternata* seeds were soaked in the EBR dilution liquid for 48 h and stirred every 12 h. Seed coating: Taking 1 kg of seeds as an example, the required TFA amount was diluted in water to 10 mL–20 mL, and the TFA liquid was mixed thoroughly with the seeds until the TFA liquid was evenly distributed on the seed surface. Each individual chemical treatment

(EBR1, EBR2, TFA1, and TFA2) went through the seed soaking and seed coating processes, and clear water replaced the chemicals in seed soaking or seed coating processes. The seeds of C1 (intragroup control) and C2 (intergroup control) were soaked with clear water with the same steps as the chemical treatment. The water temperature and drying time of the seed soaking and seed coating processes were 25 °C and 6 h, respectively. The treated seeds were broadcast sown on 20 March 2023 and then covered by 5 cm of soil, and the seeding quantity was 150 kg per 667 m^2.

2.4. Growth Determination

The plant height, leaf length, leaf width, and stem diameter of *P. ternata* were determined by a ruler or vernier calipers at the full seedling phase (May 5), vigorous growth phase (May 20), and inverted seedling phase (June 5). The vigorous growth phase refers to the period of vigorous growth of *P. ternata* plants, roughly between May 10 and May 30. The leaf area of *P. ternata* was calculated by the leaf area coefficient (0.666) method [31]:

$$\text{Leaf area} = 0.666 \times \text{leaf width} \times \text{leaf length} \quad (1)$$

For the emergence seedling ratio, 1 m^2 in the middle orientation of each plot was used as the quadrat, and the plant number in the quadrat was recorded every week after *P. ternata* began to emerge according to the methods of Hang et al. [15]. For the inverted seedling ratio, 1 m^2 in the middle orientation of each plot was used as the quadrat, the numbers of inverted live seedlings in the quadrat at the inverted seedling phase were investigated according to the methods of Cheng et al. [31].

$$\text{Emergence seedling ratio} = \text{maximum number of seedlings in the quadrat} / \text{theoretical number of seedlings in the quadrat} \quad (2)$$

$$\text{Inverted seedling ratio} = \text{live seedling number in the quadrat} / \text{live seedling number in the quadrat} + \text{inverted seedling number in the quadrat} \quad (3)$$

2.5. Electrophysiological Information Determination

Five *P. ternata* plants in the five positions of each plot were randomly chosen for monitoring their electrophysiological information 61 days after planting (May 20) according to the methods of Zhang et al. [32–34]. The third fresh fully expanded leaves of each plants were sampled and soaked in water for 30 min. Then, the water on the leaf surface was removed for measuring its capacitance (C), resistance (R), and impedance (Z) by a LCR-6300 tester (Gwinstek, Taiwan, China). Moreover, the leaf C, R and Z under different clamping forces were continuously collected and 11~13 data points were saved for each clamping force. Finally, leaf capacitive reactance (Xc) or inductive reactance (XL) were, respectively, calculated according to the following formula:

$$Xc = \frac{1}{2\pi fC} \quad (4)$$

$$\frac{1}{-XL} = \frac{1}{Z} - \frac{1}{R} - \frac{1}{Xc} \quad (5)$$

where π = 3.1416 and f = frequency.

In our previous studies, the theoretically intrinsic relationships between the clamping force and leaf R, Z, Xc, or XL were revealed as R, Z, Xc, or XL = $y + ke^{-bF}$ based on the Nernst equation [32–34]. When the clamping force is 0 (F = 0), then the intrinsic R, Z, Xc, and XL of plant leaves could be monitored as IR, IZ, IXc, or IXL = y + k. Thus, the IR, IZ, IXc, or IXL of *P. ternata* plants can be calculated by fitting the equation of clamping force and R, Z, Xc, or XL. The simple derivation of Z, R, Xc, or XL = $y + ke^{-bF}$ is as follows.

Mesophyll cell can be regarded as a concentric sphere capacitor with both inductor and resistor functions, and its ions, ion groups, and electric dipoles are used as electrolytes

for the capacitor. The concentration differences in the electrolytes that respond to R inside and outside the cell membrane obey the Nernst equation and can be expressed as follows:

$$E - E^0 = \frac{R_0 T}{n_R F_0} \ln \frac{C_i}{C_o} \quad (6)$$

where E is the electromotive force (V), E^0 is the standard electromotive force (V), R_0 is the gas constant (8.314570 J K^{-1} mol^{-1}), T is the thermodynamic temperature (K), C_i is the concentration of the electrolytes that respond to R inside the cell membrane (mol L^{-1}), C_o is the concentration of the electrolytes that respond to R outside the cell membrane (mol L^{-1}), F_0 is the Faraday constant (96485 C mol^{-1}), and n_R is the number of transferred electrolytes (mol).

When the leaf cell container is subjected to clamping force, the pressure work performed by the clamping force on leaf cells can be converted into the internal energy of the electromotive force as follows:

$$PV = aE = aE^0 + \frac{a\, R_0 T}{n_R F_0} \ln \frac{C_i}{C_o} \quad (7)$$

where P is the pressure intensity (Pa), a is the energy conversion coefficient, and V is cell volume (m^3). Formula (8) can be obtained by multi-step derivation.

$$R = \frac{f_0}{C_T} + \frac{f_0}{C_T} e^{\frac{n_R F_0 E^0}{R_0 T}} e^{\left(-\frac{d n_R F_0}{a R_0 T} F\right)} \quad (8)$$

where $d = \frac{V}{S}$, $C_T = C_o + C_i$, and f_0 is the ratio coefficient of the conversion between C_i and R. For the same leaf, the d, a, E^0, R_0, T, n_R, F_0, C_T, and f_0 in Formula (12) are constant. Let $y_0 = \frac{f_0}{C_T}$, $k_1 = \frac{f_0}{C_T} e^{\frac{n_R F_0 E^0}{R_0 T}}$, and $b_1 = \frac{d n_R F_0}{a R_0 T}$, and the intrinsic relationship of leaf R and F is $R = y_0 + k_1 e^{-b_1 F}$. When F = 0, the IR of *P. ternata* could be monitored as follows:

$$IR = y_0 + k_1 \quad (9)$$

Similar to R, the intrinsic relationship of leaf Z and F is $Z = m_0 + k_2 e^{-b_2 F}$ [32–34]. When F = 0, the IZ of *P. ternata* could be monitored as follows:

$$IZ = m_0 + k_2 \quad (10)$$

Similar to R, the intrinsic relationship of leaf Xc and F is $Xc = p_0 + k_3 e^{-b_3 F}$ [32–34]. When F = 0, the IXc of *P. ternata* could be monitored as follows:

$$IXc = p_0 + k_3 \quad (11)$$

Similar to R, the intrinsic relationship of leaf XL and F is $XL = q_0 + k_4 e^{-b_4 F}$ [32–34]. When F = 0, the IXL of *P. ternata* could be monitored as follows:

$$IXL = q_0 + k_4 \quad (12)$$

Subsequently, the intrinsic capacitance (IC) of *P. ternata* was calculated according to the following formula:

$$IC = \frac{1}{2\pi f IXc} \quad (13)$$

where $\pi = 3.1416$ and f = frequency.

According to our previous studies, the intracellular water metabolism indices of *P. ternata*, including the intracellular water-holding capacity (IWHC), intracellular water-

holding time (IWHT), and water or nutrient transfer rate (WTR or NTR), were calculated as follows:

$$\mathrm{IWHC} = \sqrt{(\mathrm{IC})^3} \quad (14)$$

$$\mathrm{IWHT} = \mathrm{IC} \times \mathrm{IZ} \quad (15)$$

$$\mathrm{WTRorNTR} = \frac{\mathrm{IWHC}}{\mathrm{IWHT}} \quad (16)$$

The nutrient transport indices of *P. ternata*, including nutrient flux per unit area (UNF), nutrient transport capacity (NTC), the active transport flow of nutrient (NAF), and nutrient active transport capacity (NAC), were calculated as follows:

$$\mathrm{UNF} = \frac{\mathrm{IR}}{\mathrm{IXc}} + \frac{\mathrm{IR}}{\mathrm{IXL}} \quad (17)$$

$$\mathrm{NTC} = \mathrm{UNF} \times \mathrm{NTR} \quad (18)$$

$$\mathrm{UAF} = \frac{\mathrm{IXc}}{\mathrm{IXL}} \quad (19)$$

$$\mathrm{NAC} = \mathrm{UAF} \times \mathrm{NTR} \quad (20)$$

Moreover, the plant metabolic activity indices of *P. ternata*, including metabolic flow (MF), metabolic rate (MR), and metabolic activity (MA), were calculated as follows:

$$\mathrm{MF} = \frac{1}{\mathrm{IR} \times \mathrm{IZ} \times \mathrm{IXc} \times \mathrm{IXL}} \quad (21)$$

$$\mathrm{MR} = \mathrm{NTR} \times \mathrm{NAC} \quad (22)$$

$$\mathrm{MA} = \sqrt[6]{\mathrm{MF} \times \mathrm{MR}} \quad (23)$$

2.6. Photosynthesis Determination

The fresh plants in same positions were chosen 61 days after planting (May 20) to monitor their photosynthesis on May 20. A SPAD-502 Plus chlorophyll meter was used to measure fully expanded leaves' SPAD index for estimating their chlorophyll content. At the same time, the portable LI-6400XT photosynthesis measurement system with photosynthetically active radiation of 1000 μmol m^{-2} s^{-1} was used to monitor fully expanded leaves' photosynthetic rate (Pn), intercellular carbon dioxide concentration (Ci), transpiration rate (Tr), stomatal conductance (Gs), and water use efficiency (WUE) at 8:00~10:00 a.m.

2.7. Resistance Determination

Moreover, the fresh plants in same positions were chosen 61 days after planting (May 20) to check their resistance levels, as described in the methods of Wang et al. [35] and Zhang et al. [36,37]. Accordingly, the thiobarbituric acid method was used to measure the malondialdehyde (MDA) content, and expressed as mmol g^{-1} FW. Additionally, the ninhydrin colorimetry, anthrone colorimetric, and Coomassie brilliant blue methods were applied for checking proline (Pro), soluble sugar, and soluble protein contents, respectively. The superoxide dismutase (SOD) activity was measured by the nitrogen blue tetrazole method, and one unit of the SOD activity was defined as the amount of enzyme that inhibited 50% of the photochemical reduction of nitrogen blue tetrazole. Meanwhile, the guaiacol, potassium permanganate titration, and cinnamic acid methods were, respectively, applied for checking superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and phenylalanine ammonia-lyase (PAL) activity, and were expressed as U g^{-1} min^{-1} FW. The hydrogen peroxide (H_2O_2) content and the rate of $O_2 \cdot^-$ production were determined according to Wang et al. [38].

2.8. Yield and Quality Determination

On September 10, *P. ternata* underground bulbs in every plot were harvested. Among them, the bulbs with a diameter $\geq$ 1.2 cm were screened as commodity materials, and other bulbs with a diameter < 1.2 cm were screened as seeds. Additionally, the weights of medicinal materials and seeds were measured by a balance. The quality of *P. ternata* bulbs was determined according to Chinese Pharmacopoeia [7] and Hang et al. [15]. Accordingly, the water, extractum, ash, and soluble sugar contents of *P. ternata* bulbs were checked by the drying, cold immersion, burning, and anthrone colorimetric methods, respectively. Meanwhile, Coomassie brilliant blue, potentiometric titration, acid dye colorimetry, and high-performance liquid chromatography methods were used for measuring their soluble protein, butanedioic acid, alkaloids, and guanosine contents, respectively.

2.9. Statistical Analysis

The significant differences in data were analyzed by the Duncan's test with one-way analysis of variance using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). In this study, each individual chemical treatment (EBR1, EBR2, TFA1, and TFA2) went through the seed soaking and seed coating processes; clear water replaced the chemicals in the seed soaking or seed coating processes. All seed soaking and seed coating processes in the experiment were processed, and so a one-factor test analysis was conducted.

3. Results

3.1. Effects of EBR and TFA on P. ternata Growth

The effects of EBR and TFA on *P. ternata* growth are displayed in Table 2. Compared with C2 (no CC), C1 (CC for 2 years) significantly ($p < 0.05$) inhibited the plant height, leaf area, and stem diameter of *P. ternata*. Compared with C1, the plant height of CC *P. ternata* treated with EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 increased significantly ($p < 0.05$). Moreover, EBR1-TFA1 and EBR1-TFA2 significantly ($p < 0.05$) promoted the leaf area of CC *P. ternata* in the full-seedling phase compared with C1; EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 significantly ($p < 0.05$) promoted its leaf area in the vigorous growth phase, and EBR1-TFA1, EBR1-TFA2, EBR1, TFA1, and TFA2 significantly ($p < 0.05$) improved its leaf area in the inverted seedling phase. Meanwhile, EBR1-TFA1 significantly ($p < 0.05$) promoted the stem diameter of CC *P. ternata* in the full-seedling and vigorous growth phases compared with C1; EBR1-TFA1, EBR1-TFA2, and TFA1 significantly ($p < 0.05$) increased its stem diameter in the inverted seedling phase. Overall, these effects on enhancing CC *P. ternata* growth were ranked as follows: EBR1-TFA1 > EBR1-TFA2 > TFA1 > TFA2 > EBR1 > EBR2. Meanwhile, their effects were not as good as C2. These findings emphasize that CC significantly ($p < 0.05$) inhibited *P. ternata* growth; seeds soaked with EBR or seeds coated with TFA could effectively enhance the plant height, leaf area, and stem diameter of CC *P. ternata*, and their combined treatment effects were more efficient.

Table 2. The effects of EBR and TFA on the plant height, leaf area, and stem diameter of *P. ternata*.

Growth Period	Treatment	Plant Height (cm)	Leaf Area (cm^2)	Stem Diameter (mm)
Full-seedling phase	EBR1-TFA1	6.06 ± 0.03^{a}	8.35 ± 0.06^{ab}	2.14 ± 0.04^{a}
	EBR1-TFA2	5.93 ± 0.04^{a}	8.34 ± 0.06^{ab}	2.09 ± 0.05^{ab}
	EBR1	5.14 ± 0.07^{c}	8.27 ± 0.07^{abc}	2.06 ± 0.04^{b}
	EBR2	5.04 ± 0.11^{c}	8.25 ± 0.12^{bc}	2.05 ± 0.04^{b}
	TFA1	5.34 ± 0.19^{b}	8.31 ± 0.14^{abc}	2.07 ± 0.04^{b}
	TFA2	5.18 ± 0.04^{c}	8.28 ± 0.04^{abc}	2.06 ± 0.04^{b}
	C1	4.86 ± 0.08^{d}	8.16 ± 0.04^{c}	2.03 ± 0.02^{b}
	C2	5.94 ± 0.07^{a}	8.42 ± 0.09^{a}	2.15 ± 0.02^{a}

Table 2. *Cont.*

Growth Period	Treatment	Plant Height (cm)	Leaf Area (cm^2)	Stem Diameter (mm)
Vigorous growth phase	EBR1-TFA1	7.58 ± 0.07^{a}	15.08 ± 0.10^{a}	2.18 ± 0.04^{a}
	EBR1-TFA2	7.05 ± 0.05^{b}	14.89 ± 0.30^{a}	2.11 ± 0.01^{ab}
	EBR1	6.58 ± 0.09^{d}	13.25 ± 0.06^{c}	2.07 ± 0.04^{b}
	EBR2	6.39 ± 0.05^{e}	12.78 ± 0.35^{d}	2.06 ± 0.07^{b}
	TFA1	6.88 ± 0.05^{c}	13.93 ± 0.32^{b}	2.09 ± 0.02^{ab}
	TFA2	6.63 ± 0.04^{d}	13.42 ± 0.29^{c}	2.07 ± 0.06^{b}
	C1	6.14 ± 0.04^{f}	11.03 ± 0.04^{e}	2.04 ± 0.10^{b}
	C2	7.69 ± 0.11^{a}	15.05 ± 0.15^{a}	2.18 ± 0.04^{a}
Inverted seedling phase	EBR1-TFA1	8.06 ± 0.05^{a}	15.08 ± 0.26^{a}	2.27 ± 0.06^{a}
	EBR1-TFA2	7.86 ± 0.10^{b}	14.92 ± 0.43^{a}	2.22 ± 0.04^{ab}
	EBR1	7.04 ± 0.03^{d}	13.98 ± 0.06^{b}	2.10 ± 0.05^{cd}
	EBR2	6.87 ± 0.08^{e}	13.26 ± 0.15^{c}	2.08 ± 0.04^{cd}
	TFA1	7.31 ± 0.04^{c}	14.43 ± 0.43^{b}	2.15 ± 0.03^{bc}
	TFA2	7.05 ± 0.08^{d}	14.03 ± 0.33^{b}	2.11 ± 0.04^{cd}
	C1	6.65 ± 0.12^{f}	12.91 ± 0.15^{c}	2.06 ± 0.07^{d}
	C2	8.05 ± 0.07^{a}	15.14 ± 0.21^{a}	2.25 ± 0.04^{a}

Data show the average values ± standard deviations, Duncan's test with one-way analysis of variance were used, and lowercase letters show significant differences at the 5% ($p < 0.05$) level.

3.2. Effects of EBR and TFA on the Emergence Seedling Ratio and Inverted Seedling Ratio of P. ternata

The inverted seedling is a physiological phenomenon of *P. ternata*; when the environmental conditions, such as allelochemicals, temperature, etc., change greatly, its aboveground stems and leaves will gradually wither [16]. Figure 1 displays the effect of EBR and TFA on the emergence seedling ratio and inverted seedling ratio of *P. ternata*. C1 significantly ($p < 0.05$) decreased the emergence seedling ratio of CC *P. ternata* and increased its inverted seedling ratio compared with C2. Compared with C1, EBR1-TFA1 significantly ($p < 0.05$) promoted the emergence seedling ratio of CC *P. ternata*; EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 effectively enhanced its emergence seedling ratio. Furthermore, the inverted seedling ratio of CC *P. ternata* treated with EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 was dramatically ($p < 0.05$) less than that treated with C1, and that treated with EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 was dramatically ($p < 0.05$) higher than that treated with C2. The results show that seeds soaked with EBR or seeds coated with TFA could effectively promote the emergence of seedlings of CC *P. ternate*, decrease the inverted seedling ratio of CC *P. ternata*, and reliably alleviate the CC stress of *P. ternata*, and their co-application was found to be more effective.

3.3. Effects of EBR and TFA on the Electrical Signals, Intracellular Water Metabolism, Nutrient Transport, and Plant Metabolic Activity of P. ternata

Plant electrical signals are the fastest physiological response to environmental stimulations or stresses. The effects of EBR and TFA on the IC, IR, IZ, IXL, and IXc of *P. ternata* are shown in Table 3. C1 significantly ($p < 0.05$) decreased the IC of CC *P. ternata* and increased its IR, IZ, IXL, and IXc compared with C2. Compared with C1, EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 significantly ($p < 0.05$) enhanced the IC of CC *P. ternata* and significantly ($p < 0.05$) decreased its IZ and IXL, and EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, and TFA1 could also significantly ($p < 0.05$) decrease its IR and IXc. Moreover, the IC of CC *P. ternata* treated with EBR1-TFA1 was significantly ($p < 0.05$) higher than that of the EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 groups, and the IR of CC *P. ternata* treated with EBR1-TFA1 was slightly lower than those treated with EBR1-TFA2, EBR1, and EBR2 and significantly ($p < 0.05$) lower than those treated with TFA1 and TFA2; the IZ, IXL, and IXc of CC *P. ternata* treated with EBR1-TFA1 were slightly lower than those treated with EBR1-TFA2 and EBR1 and significantly ($p < 0.05$) lower than those treated with EBR2, TFA1, and TFA2. These results show that the co-application of seeds soaked with EBR and seeds

coated with TFA could more effectively promote the IC of CC *P. ternata* and decrease its IR, IZ, IXL, and IXc than their applications alone, so as to more effectively promote its growth.

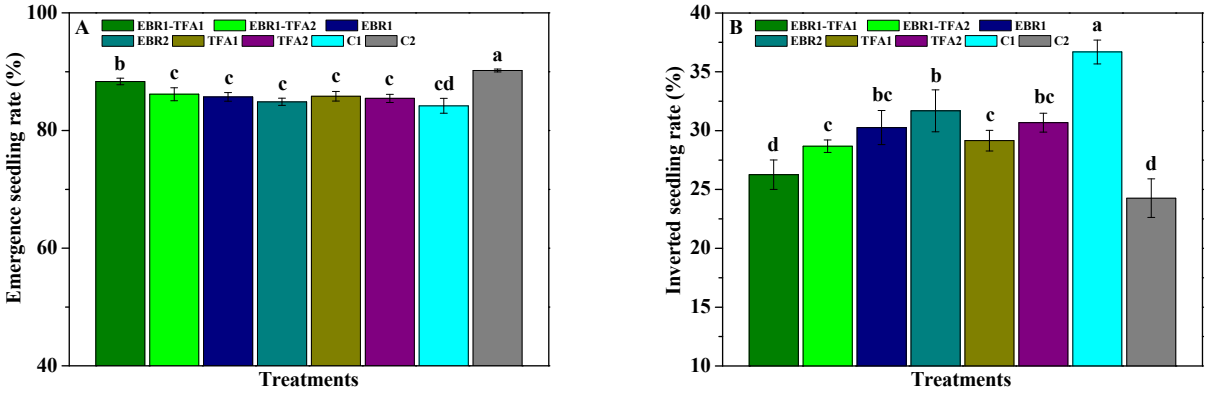

Figure 1. The effects of EBR and TFA on the emergence seedling ratio (**A**) and inverted seedling ratio (**B**) of *P. ternata*. The error bars show the standard deviations of three replicates, Duncan's test with one-way analysis of variance were used, and lowercase letters show significant differences at the 5% ($p < 0.05$) level.

Table 3. The effects of EBR and TFA on the IC, IR, IZ, IXL, and IXc of *P. ternata*.

Treatment	IC (pF)	IR (MΩ)	IZ (MΩ)	IXL (MΩ)	IXc (MΩ)
EBR1-TFA1	178.89 ± 3.56 ^{b}	2.14 ± 0.30 ^{cd}	0.30 ± 0.02 ^{ef}	0.30 ± 0.03 ^{f}	2.31 ± 0.32 ^{d}
EBR1-TFA2	160.07 ± 5.51 ^{c}	1.69 ± 0.51 ^{de}	0.33 ± 0.04 ^{ef}	0.33 ± 0.01 ^{ef}	1.87 ± 0.32 ^{de}
EBR1	147.40 ± 4.98 ^{d}	2.28 ± 0.42 ^{cd}	0.34 ± 0.03 ^{de}	0.36 ± 0.01 ^{de}	2.49 ± 0.46 ^{cd}
EBR2	132.13 ± 2.16 ^{e}	3.03 ± 0.22 ^{bc}	0.40 ± 0.02 ^{cd}	0.40 ± 0.01 ^{d}	3.24 ± 0.30 ^{bc}
TFA1	109.70 ± 4.99 ^{f}	3.56 ± 0.48 ^{b}	0.46 ± 0.04 ^{c}	0.49 ± 0.02 ^{c}	3.58 ± 0.30 ^{b}
TFA2	89.41 ± 1.18 ^{g}	5.09 ± 0.17 ^{a}	0.58 ± 0.02 ^{b}	0.59 ± 0.01 ^{b}	5.40 ± 0.20 ^{a}
C1	79.45 ± 7.15 ^{h}	5.76 ± 1.01 ^{a}	0.70 ± 0.06 ^{a}	0.67 ± 0.06 ^{a}	5.98 ± 0.85 ^{a}
C2	217.05 ± 1.58 ^{a}	1.01 ± 0.46 ^{e}	0.26 ± 0.06 ^{f}	0.24 ± 0.01 ^{g}	1.17 ± 0.46 ^{e}

Data show the average values ± standard deviations, Duncan's test with one-way analysis of variance were used, and lowercase letters show significant differences at the 5% ($p < 0.05$) level.

The effects of EBR and TFA on the IWHC, IWHT, WTR or NTR, UNF, NTC, UAC, MF, MR, and MA of *P. ternata* are shown in Figure 2. Compared with C2, C1 significantly ($p < 0.05$) decreased the IWHC, NTC, UAC, MF, MR, and MA of CC *P. ternata*. For the intracellular water metabolism of CC *P. ternata*, EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 significantly ($p < 0.05$) enhanced the IWHC of CC *P. ternata* compared with C2 and significantly ($p < 0.05$) decreased its WTR or NTR. Moreover, the IWHC of CC *P. ternata* treated with EBR1-TFA1 was significantly ($p < 0.05$) higher than that treated with EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2, and its WTR or NTR after treatment with EBR1-TFA1 was significantly ($p < 0.05$) higher than that of plants treated with EBR1, EBR2, TFA1, and TFA2. For the nutrient transport status of CC *P. ternata*, EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, and TFA1 significantly ($p < 0.05$) enhanced the NTC and NAC of CC *P. ternata* compared with C2, and its NTC after treatment with EBR1-TFA1 was significantly ($p < 0.05$) higher than that of the EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 groups; its UAC after treatment with EBR1-TFA1 or EBR1-TFA2 was significantly ($p < 0.05$) higher than that of the EBR2, TFA1, and TFA2 groups. For the metabolic activity of CC *P. ternata* plants, EBR1-TFA1, EBR1-TFA2, and EBR1 significantly ($p < 0.05$) enhanced the MF of CC *P. ternata* compared with C2, and EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, and TFA1 also significantly ($p < 0.05$) promoted its MR and MA. Meanwhile, its MF, MR, and MA after treatment with EBR1-TFA1 or EBR1-TFA2 were significantly ($p < 0.05$) higher than those of the EBR2, TFA1, and TFA2 groups and slightly than that of the EBR1 group. The results demonstrate that seeds soaked with EBR or seeds coated with TFA effectively enhanced the intracellular

water metabolism, nutrient transport, and plant metabolic activity of CC *P. ternata* and improved its healthy growth, and their co-application had a more effective function.

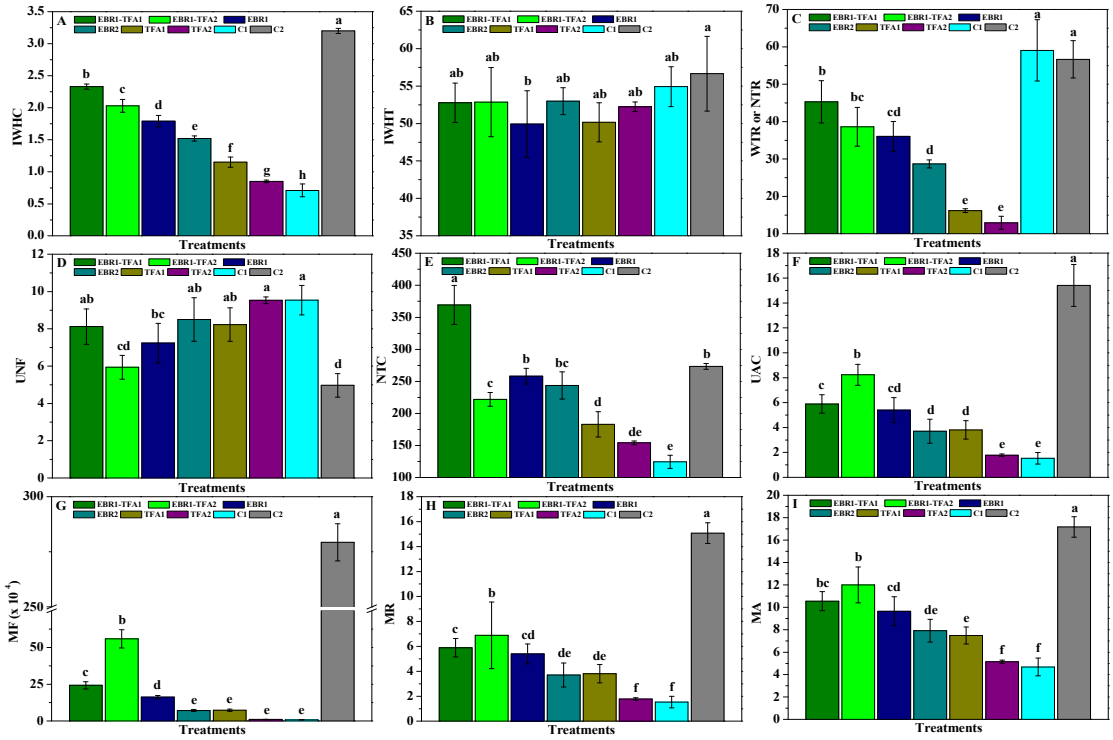

Figure 2. The effects of EBR and TFA on the IWHC (**A**), IWHT (**B**), WTR or NTR (**C**), UNF (**D**), NTC (**E**), UAC (**F**), MF (**G**), MR (**H**), and MA (**I**) of *P. ternata*. The error bars show the standard deviations of three replicates, Duncan's test with one-way analysis of variance were used, and lowercase letters show significant differences at the 5% ($p < 0.05$) level.

3.4. Effects of EBR and TFA on P. ternata Photosynthesis

Figure 3 shows the effects of EBR and TFA on the chlorophyll, Pn, Ci, Tr, and Gs contents and WUE of *P. ternata* leaves. C1 dramatically ($p < 0.05$) decreased the chlorophyll, Pn, Ci, Tr, and Gs contents and WUE of *P. ternata* leaves compared with C2. Compared with C1, EBR1-TFA1, EBR1-TFA2, and TFA1 dramatically ($p < 0.05$) improved the chlorophyll content in CC *P. ternata*, and EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 significantly ($p < 0.05$) strengthened its Pn, Ci, Tr, and Gs contents and WUE. Meanwhile, the chlorophyll, Pn, Ci, Tr, and Gs contents and WUE of CC *P. ternata* treated with EBR1-TFA1 were slightly lower than those treated with C2, and its chlorophyll, Ci, and Tr contents when treated with EBR1-TFA2 were dramatically ($p < 0.05$) lower than those treated with C2; in addition, its chlorophyll, Pn, Ci, Tr, and Gs contents when treated with EBR1, EBR2, TFA1, and TFA2 were significantly ($p < 0.05$) lower than those of the C2 group. Correspondingly, these effects on enhancing the chlorophyll, Pn, Ci, Tr, and Gs contents in CC *P. ternata* were also ranked as follows: EBR1-TFA1 > EBR1-TFA2 > TFA1 > TFA2 > EBR1 > EBR2. The findings presented here demonstrate that seeds soaked with EBR or seeds coated with TFA effectively enhanced the chlorophyll, Pn, Ci, Tr, and Gs contents and WUE of CC *P. ternata* and evidently promoted its healthy growth, and their co-application could more reliably ameliorate its photosynthesis.

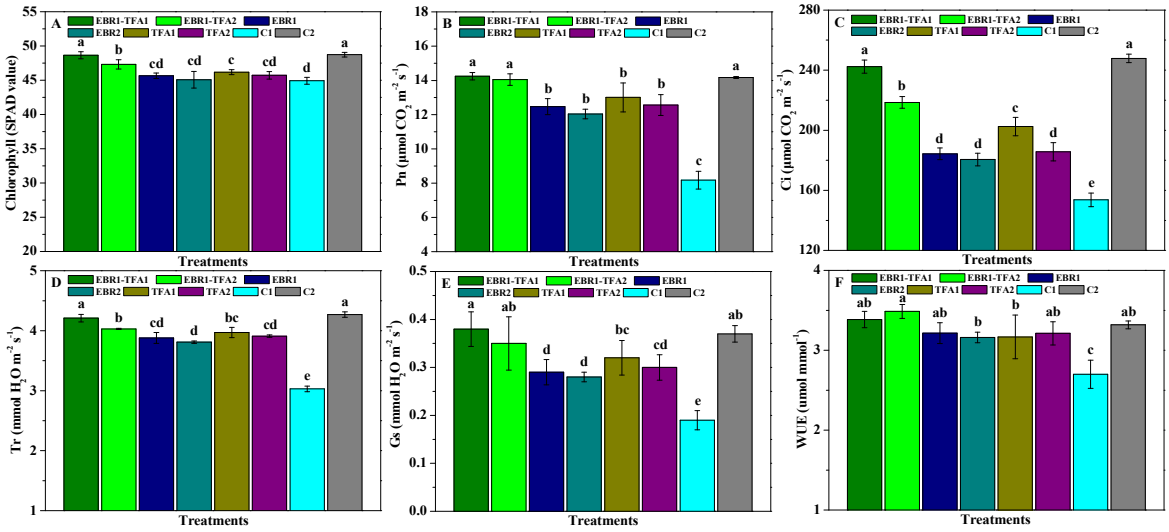

Figure 3. The effects of EBR and TFA on the chlorophyll (**A**), Pn (**B**), Ci (**C**), Tr (**D**), Gs (**E**), and WUE (**F**) of *P. ternata*. The error bars show the standard deviations of three replicates, Duncan's test with one-way analysis of variance were used, and lowercase letters show significant differences at the 5% ($p < 0.05$) level.

3.5. Effects of EBR and TFA on P. ternata Resistance

The effects of EBR and TFA on the MDA, H_2O_2, Pro, soluble sugar, and soluble protein contents, and the rate of $O_2 \cdot^{-1}$ production in *P. ternata* are displayed in Figure 4. C1 significantly ($p < 0.05$) increased the MDA, H_2O_2, Pro, and soluble sugar contents and the rate of $O_2 \cdot^{-1}$ production in *P. ternata* compared to C2 and reduced its soluble protein content. EBR1-TFA1 significantly ($p < 0.05$) decreased the MDA, H_2O_2, Pro, and soluble sugar contents and the rate of $O_2 \cdot^{-1}$ production in *P. ternata* compared to C1 and increased its soluble protein content. Compared to C1, EBR1-TFA2 also caused significant ($p < 0.05$) decreases in the MDA, H_2O_2, and Pro contents and the rate of $O_2 \cdot^{-1}$ production in *P. ternata*. The H_2O_2 and Pro contents in *P. ternata* treated with EBR1, EBR2, TFA1, and TFA2 were also significantly ($p < 0.05$) less than those treated with C1. These results reveal that the co-application of seeds soaked with EBR and seeds coated with TFA could more effectively decrease the MDA, H_2O_2, Pro, and soluble sugar contents and the rate of $O_2 \cdot^{-1}$ production in *P. ternata* and increase its soluble protein content than their applications alone and then effectively ameliorate the stress resistance of CC *P. ternata*.

The effects of EBR and TFA on the SOD, POD, CAT, and PAL activities in *P. ternata* are displayed in Figure 4. C1 significantly ($p < 0.05$) reduced the SOD, POD, CAT, and PAL activities in *P. ternata* compared to C2. Compared with C1, EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 dramatically ($p < 0.05$) improved the SOD, POD, and PAL activities in CC *P. ternata*, and EBR1-TFA1 significantly ($p < 0.05$) enhanced its CAT activity. In addition, the SOD, POD, and PAL activities in CC *P. ternata* treated with EBR1, EBR2, TFA1, and TFA2 were significantly ($p < 0.05$) less than those treated with C2, and the SOD, POD, CAT, and PAL activities in CC *P. ternata* treated with EBR1-TFA1 and EBR1-TFA2 were slightly lower than those treated with C2. These results reveal that the co-application of seeds soaked with EBR and seeds coated with TFA could more effectively improve the SOD, POD, CAT, and PAL activities of CC *P. ternata*, further enhancing its stress resistance.

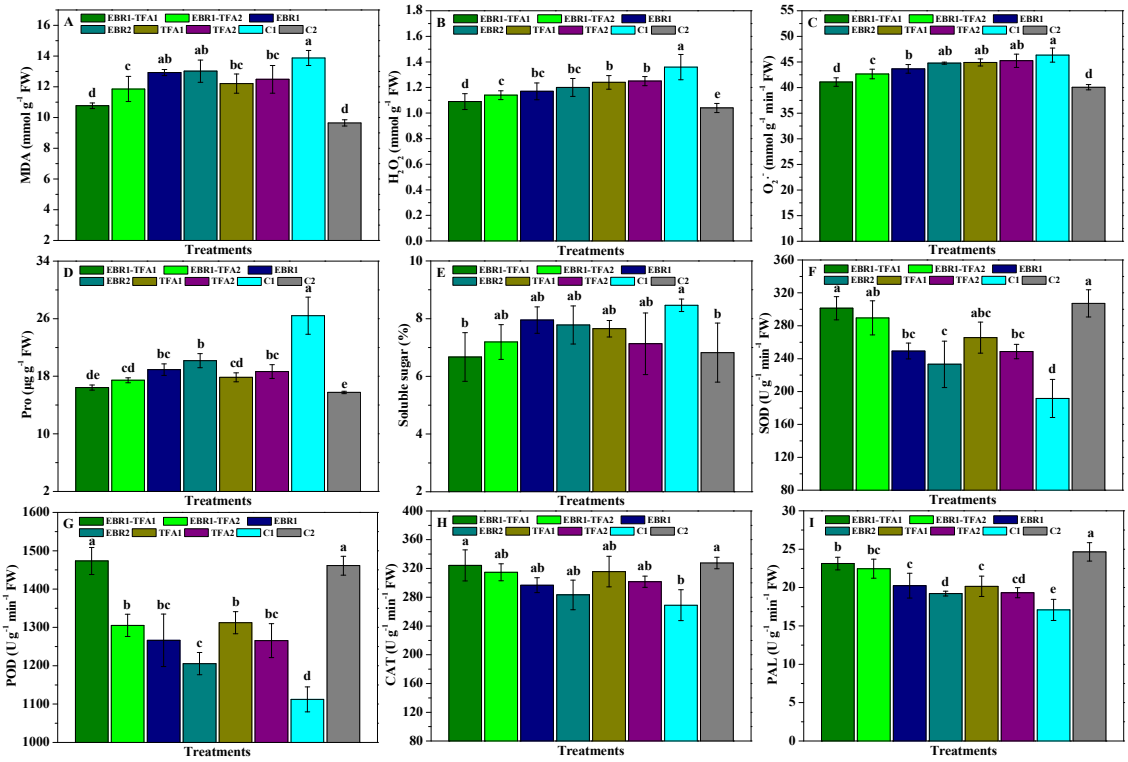

Figure 4. The effects of EBR and TFA on the MDA (**A**), H_2O_2 (**B**), Pro (**D**), and soluble sugar (**E**) contents, the rate of $O_2 \cdot^{-1}$ production (**C**), and SOD (**F**), POD (**G**), CAT (**H**), and PAL (**I**) activities in *P. ternata*. The error bars show the standard deviations of three replicates, Duncan's test with one-way analysis of variance were used, and lowercase letters show significant differences at the 5% ($p < 0.05$) level.

3.6. Effects of EBR and TFA on P. ternata Quality

The effects of EBR and TFA on the water, extractum, ash, and soluble sugar contents of *P. ternata* bulbs are shown in Figure 5. C1 dramatically ($p < 0.05$) decreased the water, extractum, ash, and soluble sugar contents of CC *P. ternata* bulbs compared to C2. Compared with C1, EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 dramatically ($p < 0.05$) enhanced the water, extractum, ash, and soluble sugar contents of CC *P. ternata* bulbs. Additionally, the water, extractum, ash, and soluble sugar contents in CC *P. ternata* bulbs treated with EBR1, EBR2, TFA1, and TFA2 were evidently lower than those of bulbs treated with EBR1-TFA1, EBR1-TFA2, and C2, and those treated with EBR1-TFA1, EBR1-TFA2, and C2 showed no significant differences. These results show that the co-application of seeds soaked with EBR and seeds coated with TFA markedly increased the water, extractum, ash, and soluble sugar contents of CC *P. ternata* bulbs.

The effects of EBR and TFA on the soluble protein, butanedioic acid, alkaloid, and guanosine contents in *P. ternata* bulbs are displayed in Figure 5. Compared to C2, C1 also significantly ($p < 0.05$) decreased the soluble protein, butanedioic acid, alkaloid, and guanosine contents in CC *P. ternata* bulbs. Compared with C1, EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 dramatically ($p < 0.05$) increased the soluble protein and alkaloid contents of CC *P. ternata* bulbs, and EBR1-TFA1, EBR1-TFA2, EBR1, TFA1, and TFA2 significantly ($p < 0.05$) increased its butanedioic acid content; EBR1-TFA1 and EBR1-TFA2 significantly ($p < 0.05$) improved its guanosine content. Furthermore, the soluble protein content of CC *P. ternata* bulbs treated with EBR1, EBR2, TFA1, and TFA2 was significantly ($p < 0.05$) less than that of the C2 group, and the butanedioic acid, alkaloid, and guanosine contents of CC *P. ternata* bulbs treated with EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 were significantly ($p < 0.05$) lower than that of the C2 group. These results indicate that the co-application of seeds soaked with EBR and seeds coated with

TFA effectively improved the soluble protein, butanedioic acid, alkaloid, and guanosine contents of CC *P. ternata* bulbs. Overall, the co-application of seeds soaked with EBR and seeds coated with TFA effectively ameliorated the medicinal quality of CC *P. ternata*.

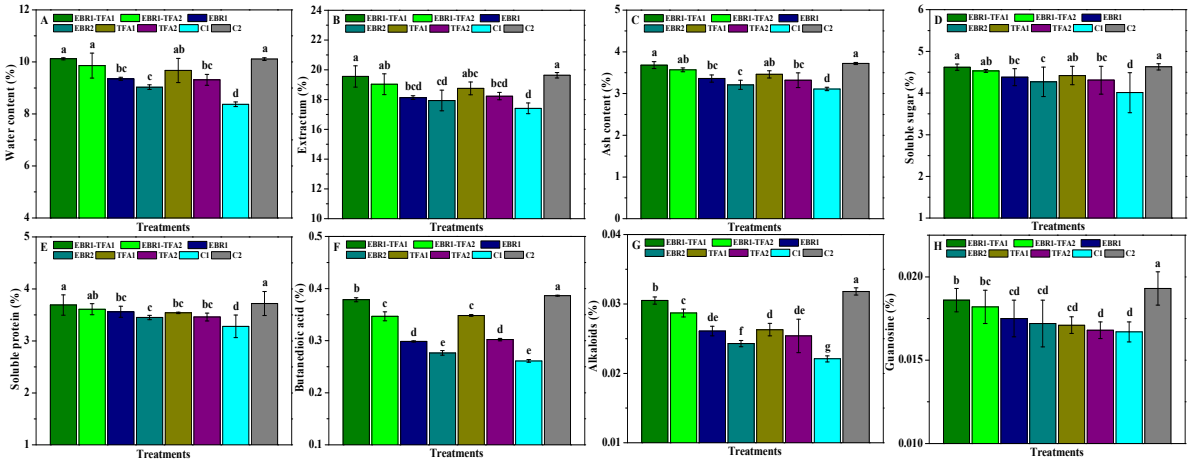

Figure 5. The effects of EBR and TFA on the water (**A**), extractum (**B**), ash (**C**), soluble sugar (**D**), soluble protein (**E**), butanedioic acid (**F**), alkaloid (**G**), and guanosine (**H**) contents in *P. ternata*. The error bars show the standard deviations of three replicates, Duncan's test with one-way analysis of variance were used, and lowercase letters show significant differences at the 5% ($p < 0.05$) level.

3.7. Effects of EBR and TFA on P. ternata Yield

The effects of EBR and TFA on the medicinal material weight, seed weight, and total yield of *P. ternata* are displayed in Table 4. Compared with C2, C1 also significantly ($p < 0.05$) reduced the medicinal material weight, seed weight, and total yield of *P. ternata*. Compared to C1, EBR1-TFA1, EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 significantly ($p < 0.05$) enhanced the medicinal material weight, seed weight, and total yield of CC *P. ternata*, and they were also ranked as follows: EBR1-TFA1 > EBR1-TFA2 > TFA1 > TFA2 > EBR1 > EBR2. Furthermore, the medicinal material weight, seed weight, and total yield of CC *P. ternata* treated with EBR1-TFA1 were slightly lower than those of the C2 group, and those treated with EBR1-TFA2, EBR1, EBR2, TFA1, and TFA2 were significantly ($p < 0.05$) lower than those treated with C2. The results indicate that the co-application of seeds soaked with EBR and seeds coated with TFA could satisfactorily alleviate *P. ternata* cropping obstacles and increase its yield.

Table 4. The effects of EBR and TFA on the medicinal material weight, seed weight, and total yield of *P. ternata*.

Treatment	Medicinal Material Weight (kg per 667 m^2)	Seed Weight (kg per 667 m^2)	Total Yield (kg per 667 m^2)
EBR1-TFA1	52.16 ± 3.72 ^{a}	244.87 ± 10.37 ^{a}	192.71 ± 12.10 ^{a}
EBR1-TFA2	48.86 ± 3.41 ^{b}	216.36 ± 12.94 ^{b}	167.50 ± 10.98 ^{b}
EBR1	43.82 ± 6.12 ^{b}	200.18 ± 17.61 ^{bc}	156.36 ± 13.41 ^{b}
EBR2	41.65 ± 2.65 ^{b}	193.65 ± 10.37 ^{c}	152.00 ± 9.20 ^{b}
TFA1	47.53 ± 1.65 ^{b}	211.27 ± 5.94 ^{bc}	163.74 ± 7.05 ^{b}
TFA2	44.68 ± 4.35 ^{b}	202.56 ± 1.96 ^{bc}	157.88 ± 3.74 ^{b}
C1	28.17 ± 3.47 ^{c}	125.26 ± 10.10 ^{d}	97.09 ± 9.61 ^{c}
C2	54.65 ± 1.55 ^{a}	247.42 ± 4.30 ^{a}	192.77 ± 5.85 ^{a}

Data show the average values ± standard deviations, Duncan's test with one-way analysis of variance were used, and lowercase letters show significant differences at the 5% ($p < 0.05$) level.

4. Discussion

CC seriously limits *P. ternata* growth and frequently results in economic losses of 40%~60% [9,10]. Xiao [12] reported that CC of *P. ternata* for 2 years resulted in a worse obstacle/problem compared with CC for 1, 3, 4, and 5 years. The results show that *P. ternata* with CC for 2 years was in a stress state of CC; its growth, photosynthesis, quality, and yield were seriously inhibited compared with non-CC *P. ternata.* As a plant growth promoter, EBR has many beneficial functions in plants, including cell division, cell elongation, seed germination, root development, flowering, senescence, and stress responses [16–20]. Thiamethoxam·flutolanil·azoxystrobin was registered as a seed coating agent that can protect seeds from various pathogens and pests in soils. In this study, seeds soaked with EBR or seeds coated with TFA could effectively enhance the plant height, leaf area, and stem diameter of CC *P. ternata,* promote its emergence seedling ratio, and decrease its inverted seedling ratio, and their co-application was found to be more efficient. These excellent results may be attributed to the synergistic effects between the growth-enhancing roles of EBR and the prevention and control roles of TFA.

After the CC of *P. ternata* in the same land, its medicinal quality deteriorated and bulb yield is constantly decreased [9,10], while good growth and development determine its high quality and yield. Brassinolide is recognized as the sixth-largest plant hormone after auxin, abscisic acid, cytokinin, gibberellin, and salicylic acid and plays important roles in improving the chlorophyll content, promoting photosynthesis, enhancing seed development, root growth, blooming, and maturation, as well as ameliorating quality and increasing yield [39–41]. In this study, under the stress of CC, the co-application of seeds soaked with EBR and seeds coated with TFA could not only promote the growth, photosynthesis, and resistance of *P. ternata* but also significantly enhance its medicinal quality (i.e., water, extractum, ash, soluble sugar, soluble protein, butanedioic acid, alkaloid, and guanosine contents) and bulb yield (i.e., medicinal material weight, seed weight, and total yield). This is closely related to the growth-enhancing roles of EBR in *P. ternata* and the preventative and control roles of TFA against diseases and pests.

Plants electrophysiological activities almost run through all life processes, and are considered as the fastest physiological response to environmental stimulations [42–44]. Generally, a mesophyll cell is equivalent to a concentric sphere capacitor with both an inductor and resistor function, and its ions, ion groups, and electric dipoles are capacitor electrolytes [32–34,45,46]. An abiotic or biotic stress, including CC, drought, diseases and insect pests, can directly or indirectly evoke structural, composition and ion permeability changes in plant cells, causing dramatic changes in a plant's electrophysiological activities [32–34,42–44]. Previously, C, R, Z, Xc, and XL were the most common electrical signals used to evaluate various plants' physiological statuses. For example, Zhang et al. [47] firstly defined leaf tensity based on leaf C to represent plant drought resistance, Xing et al. [48] reported that Z provides more reliable plant water information and defined water dissipation rates based on Z, and our previous report found that IXc and IXL could be used to manifest the relatively composition characteristic of cell membrane proteins [34]. Moreover, the intracellular water metabolism, nutrient transport, and plant metabolic activity parameters based on the plant's electrophysiological information in our previous studies could accurately, reliably, and rapidly manifest its physiological processes [32–34]. In this study, seeds soaked with EBR or seeds coated with TFA effectively enhanced the IC, intracellular water metabolism, nutrient transport, and plant metabolic activity of CC *P. ternata* and decrease its IR, IZ, IXL, and IXc, and their co-application had a more effective function. These results commendably characterized the physiological activities of CC *P. ternata*, supported the research conclusions of the above agronomic trait, and expanded the application of a plant's electrophysiological information in plant stress biology.

EBR plays a crucial role in stress responses, including drought, salinity, heavy metals, and extreme temperature [49]. For example, Kolomeichuk et al. [22] found that EBR could enhance the photosynthetic pigments, electron transport, and photosystem II maximum of *Solanum tuberosum* L. under salt stress. Junior et al. [50] reported that EBR increased

the Pn, Tr, and Gs contents of *Eucalyptus urophylla* for involvement in the drought stress response. Moreover, many studies have shown that the occurrence and development of CC obstacles in *P. ternata* are directly or indirectly driven by rhizosphere allelochemicals such as phenolic acids, quinones, ketones, and phenols [9,14,51]. In this work, under CC stress (or allelochemical stress), seeds soaked with EBR or seeds coated with TFA effectively enhanced the chlorophyll, Pn, Ci, Tr, and Gs contents and WUE of CC *P. ternate*, and their co-application could more reliably ameliorate its photosynthesis. These results are similar to the above research conclusions and expand the application of EBR and TFA.

Soluble sugar and Pro participate in regulating the osmotic potential of plants under stress, and MDA can directly reflect the peroxidation level of the cell plasma membrane [52]. The harm caused by reactive oxygen species can be alleviated by SOD and CAT, and H_2O_2 in lignin biosynthesis can be catalytically decomposed by POD; in addition, PAL plays an important role in the metabolism of secondary substances (such as lignin) in plants [52]. These indicators are closely related to the stress resistance of plants. Rattan et al. [53] showed that EBR reduced the MDA content of *Zea mays* L. seedlings and significantly increased their Pro content to overcome oxidative damage under salt stress. Soares et al. [17] found that EBL could increase the activities of SOD, CAT, POD, PAL, and other antioxidant enzymes in *Brassica juncea* to overcome the toxic effects of lead. In this work, under CC stress, the co-application of seeds soaked with EBR and seeds coated with TFA could more effectively decrease the MDA, H_2O_2, Pro, and soluble sugar contents and the rate of $O_2 \cdot^{-1}$ production in CC *P. ternata* than their applications alone and enhance its SOD, POD, CAT, and PAL activities. These results demonstrate that the co-application of seeds soaked with EBR and seeds coated with TFA could reliably ameliorate the stress resistance of CC *P. ternata.*

The application dosages of EBR and TFA used in this study were both recommended by the Chinese Ministry of Agriculture; in addition, the safe interval of 173 days for medicinal materials was also quite long. In this way, the number of safety issues regarding medicinal materials caused by EBR or TFA was extremely low or non-existent. Overall, this work emphasizes that seeds soaked with 0.004% EBR AS in a 1000-times dilution liquid + seeds coated with 24% TFA FC at 1.6 mL kg^{-1} is a practicable formula for alleviating the CC stress of *P. ternata* and ameliorating its growth, photosynthesis, resistance, quality, and yield.

5. Conclusions

In summary, seeds soaked with EBR or seeds coated with TFA could effectively enhance the plant growth of CC *P. ternata*, promote its emergence seedling ratio, and decrease its inverted seedling ratio, and the effects of their combined application were more efficient. Additionally, their co-application effectively enhanced the IC, intracellular water metabolism, nutrient transport, and plant metabolic activity of CC *P. ternata* and decreased its IR, IZ, IXL, and IXc. Meanwhile, their co-application could reliably ameliorate the photosynthesis and resistance of CC *P. ternata*. Furthermore, their co-application could also effectively enhance CC *P. ternata*'s medicinal quality and bulb yield. This study highlights that the co-application of seeds soaked with EBR and seeds coated with TFA can be recommended as a novel and practicable measure in the efficacious alleviation of CC stress in *P. ternata.*

Author Contributions: Conceptualization, C.Z. and Y.L.; Methodology, B.T., C.T. and J.L.; Software, B.T., C.T., Q.W. and Y.S.; Formal analysis, Q.W.; Investigation, B.T., C.T., J.L. and Q.W.; Resources, Wenhao Feng; Data curation, B.T., C.T. and J.L.; Writing—original draft, B.T.; Writing—review & editing, C.Z.; Supervision, W.F., Y.S. and Y.L.; Funding acquisition, C.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (No. 32360023), the Science–Technology Support Program of Guizhou Province [No. QKHZC(2023)Yiban074], the Earmarked Fund for Guizhou Modern Agriculture Research System (No. GZRNCYJSTX2024-4), the Scientific Research University Project of Education Department in Guizhou Province (Youth Project) [No. Qianjiaoji (2022) 196], the Humanities and Social Science Research Project of Guizhou Provincial University (No. 2024RW87), the Guizhou Provincial Foundation for Excellent Scholars Program

[No. GCC(2023)076], the Youth Science and Technology Talent Growth Project of Guizhou Medical University (No. 22QNRC15), and the High-level Talent Fund Project of Guizhou Medical University (No. Xiaobohe (2022) 007).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Liu, Y.; Newmaster, S.; Wu, X.; Liu, Y.; Long, C. *Pinellia* hunanensis (*Araceae*), A New Species Supported by Morphometric Analysis and DNA Barcoding. *Phytotaxa* **2013**, *130*, 1–13. [CrossRef]
2. Ji, X.; Huang, B.; Wang, G.; Zhang, C. The Ethnobotanical, Phytochemical and Pharmacological Profile of the Genus *Pinellia*. *Fitoterapia* **2014**, *93*, 1–17. [CrossRef] [PubMed]
3. Yu, H.; Pan, Y.; Wu, H.; Ge, X.; Cai, B. The Alum-processing Mechanism Attenuating Toxicity of *Araceae Pinellia ternata* and *Pinellia pedatisecta*. *Arch. Pharmacal Res.* **2015**, *38*, 1810–1821. [CrossRef] [PubMed]
4. Zhang, Z.; Zhao, Y.; Cheng, X.; Lin, R.; Dai, Z.; Zhou, C. Metabonomic Study of Biochemical Changes in the Rat Urine Induced by *Pinellia ternata* (thunb.) berit. *J. Pharm. Biomed. Anal.* **2013**, *85*, 186–193. [CrossRef] [PubMed]
5. Chen, J.; Cui, G.; Liu, J.; Tan, R. Pinelloside, An Antimicrobial Cerebroside from *Pinellia ternata*. *Phytochemistry* **2003**, *64*, 903–906. [CrossRef] [PubMed]
6. Zhang, Y.; Xia, Z.; Li, Q.; Gui, G.; Zhao, G.; Lin, L. Surface Controlled Electrochemical Sensing of Chlorpyrifos in *Pinellia ternate* Based on a One Step Synthesis of Palladium-reduced Graphene Nanocomposites. *J. Electrochem. Soc.* **2016**, *164*, B48–B53. [CrossRef]
7. Chinese Pharmacopoeia Commission. *General Principles of Four Parts of Chinese Pharmacopoeia 2020*; China Medical Science and Technology Press: Beijing, China, 2019.
8. Li, X.; Zhang, X.; Yang, W.; Guo, L.; Huang, L.; Li, X.; Gao, W. Preparation and Characterization of Native and Autoclaving-cooling Treated *Pinellia ternate* Starch and Its Impact on Gut Microbiota. *Int. J. Biol. Macromol.* **2021**, *182*, 1351–1361. [CrossRef] [PubMed]
9. He, Z.; Chen, H.; Liang, L.; Dong, J.; Liang, Z.; Zhao, L. Alteration of Crop Rotation in Continuous *Pinellia ternate* Cropping Soils Profiled via Fungal ITS Amplicon Sequencing. *Lett. Appl. Microbiol.* **2019**, *68*, 522–529. [CrossRef] [PubMed]
10. Tang, C.; Luo, F.; Zhao, Z.; Hang, Y.; Wang, H.; Cheng, S.; Liu, H. The Allelopathy of *Pinellia ternata* Decomposed Liquid on 8 Crops and Composition of Allelochemicals. *J. Nucl. Agric. Sci.* **2018**, *32*, 1639–1648.
11. Tang, C.; Lei, Q.; Luo, F.; Zhao, Z.; Hang, Y.; Wang, H.; Luo, C.; Cheng, S. Allelopathic Effects of *Pinellia ternata* Extracts on Eight Crops. *Guizhou Agric. Sci.* **2019**, *47*, 31–37.
12. Xiao, Y.; Luo, F. Analysis of Transcriptome Response to 4-methyl-2,6-di-tert-butylphenol in *Pinellia ternate* root. *Mol. Plant Breed.* **2022**, 1–22. Available online: http://kns.cnki.net/kcms/detail/46.1068.S.20220225.0902.006.html (accessed on 26 February 2022).
13. He, Z.; Mao, R.; Dong, J.; Liang, Z.; Zhang, H.; Liu, L. Remediation of Deterioration in Microbial Structure in Continuous *Pinellia ternata*, Cropping Soil by Crop Rotation. *Can. J. Microbiol.* **2019**, *65*, 282–295. [CrossRef] [PubMed]
14. Zhao, Y.; Qin, X.; Tian, X.; Yang, T.; Huang, J. Effects of Continuous Cropping of *Pinellia ternata* (Thunb.) Breit. on Soil Physicochemical Properties, Enzyme Activities, Microbial Communities and Functional Genes. *Chem. Biol. Technol. Agric.* **2021**, *8*, 43. [CrossRef]
15. Hang, Y.; Luo, F.; Zhao, Z.; Tang, C.; Cheng, S.; Lin, J. Comprehensive Assessment of *Pinellia ternata* with Different Intercropping Patterns on Yield and Quality. *North. Hortic.* **2018**, *13*, 132–140.
16. Dehghana, M.; Balouchib, H.; Yadavib, A.; Zare, E. Improve Wheat (*Triticum aestivum*) Performance by Brassinolide Application Under Different Irrigation Regimes. *S. Afr. J. Bot.* **2020**, *130*, 259–267. [CrossRef]
17. Soares, T.; Dias, D.; Oliveira, A.; Ribeiro, D.M.; Dias, L. Exogenous Brassinosteroids Increase Lead Stress Tolerance in Seed Germination Seedling Growth of *Brassica juncea* L. *Ecotoxicol. Environ. Saf.* **2020**, *193*, 110296. [CrossRef] [PubMed]
18. Demissie, Z.A.; Huang, F.; Song, H.; Todd, A.T.; Loewen, M.C. Barley "uzu" Wheat "uzu-like" Brassinosteroid Receptor BRI1 Kinase Domain Variations Modify Phosphorylation Activity in Vitro. *Biochemistry* **2020**, *59*, 2986–2997. [CrossRef] [PubMed]
19. Nolan, T.M.; Vukašinovi´c, N.; Liu, D.; Yin, Y.H. Brassinosteroids: Multidimensional Regulators of Plant Growth, Development, Stress Responses. *Plant Cell* **2020**, *32*, 295–318. [CrossRef] [PubMed]
20. Hu, D.; Wei, L.; Liao, W. Brassinosteroids in Plants: Crosstalk with Small-Molecule Compounds. *Biomolecules* **2021**, *11*, 1800. [CrossRef] [PubMed]
21. Li, T.; Lei, W.; He, R.; Tang, X.; Zhang, D. Brassinosteroids Regulate Root Meristem Development by Mediating BIN2-UPB1 Module in *Arabidopsis*. *PLoS Genet.* **2020**, *16*, e1008883. [CrossRef] [PubMed]
22. Kolomeichuk, L.V.; Efimova, M.V.; Zlobin, I.E.; Kreslavski, V.D.; Allakhverdiev, S.I. 24-Epibrassinolide Alleviates the Toxic eEffects of NaCl on Photosynthetic Processes in Potato Plants. *Photosynth. Res.* **2020**, *146*, 151–163. [CrossRef] [PubMed]
23. Fu, J.; Sun, P.; Luo, Y.; Zhou, H.; Gao, J.; Zhao, D.; Pubu, Z.; Liu, J.; Hu, T. Brassinosteroids Enhance Cold Tolerance in *Elymusnutans* via Mediating Redox Homeostasis Proline Biosynthesis. *Environ. Exp. Bot.* **2019**, *167*, 103831. [CrossRef]
24. Li, L.; Yin, S.; Pan, W.; Wang, F.; Fan, J. Comprehensive Metabolome and Growth Effects of Thiamethoxam Uptake and Accumulation from Soil on Pak Choi. *Food Chem.* **2024**, *433*, 137286. [CrossRef] [PubMed]

25. Nauen, R.; Jeschke, P.; Schindler, M.; Elbert, A. Overview of the Status and Global Strategy for Neonicotinoids. *J. Agr. Food Chem.* **2011**, *59*, 2897–2908. [CrossRef]
26. Zhao, C.; Li, S.; Ma, Z.; Wang, W.; Gao, L.; Han, C.; Yang, A.; Wu, X. Anastomosis Groups and Mycovirome of *Rhizoctonia* Isolates Causing Sugar Beet Root and Crown Rot and Their Sensitivity to Flutolanil, Thifluzamide, and Pencycuron. *J. Fungi* **2023**, *9*, 545. [CrossRef] [PubMed]
27. Rodriguez-Morelos, V.H.; Calonne-Salmon, M.; Bremhorst, V.; Garcés-Ruiz, M.; Declerck, S. Fungicides With Contrasting Mode of Action Differentially Affect Hyphal Healing Mechanism in *Gigaspora* sp. and *Rhizophagus irregularis*. *Front. Plant Sci.* **2021**, *12*, 642094. [CrossRef] [PubMed]
28. Wang, R.; Huang, N.; Ji, J.; Chen, C. An Integrated Approach for Evaluating the Interactive Effects Between Azoxystrobin and Ochratoxin A: Pathway-based in Vivo Analyses. *Pestic. Biochem. Physiol.* **2023**, *195*, 105556. [CrossRef]
29. Dong, J.; Huang, M.; Guo, H.; Zhang, J.; Tan, X.; Wang, D. Ternary Mixture of Azoxystrobin, Boscalid and Pyraclostrobin Disrupts the Gut Microbiota and Metabolic Balance of Honeybees (*Apis cerana cerana*). *Int. J. Mol. Sci.* **2023**, *24*, 5354. [CrossRef] [PubMed]
30. Fang, A.; Zhang, R.; Qiao, W.; Peng, T.; Qin, Y.; Wang, J.; Tian, B.; Yu, Y.; Sun, W.; Yang, Y.; et al. Sensitivity Baselines, Resistance Monitoring, and Molecular Mechanisms of the Rice False Smut Pathogen Ustilaginoidea virens to Prochloraz and Azoxystrobin in Four Regions of Southern China. *J. Fungi* **2023**, *9*, 832. [CrossRef] [PubMed]
31. Chen, F.; Li, Q.; Su, Y.; Lei, Y.; Zhang, C. Chitosan Spraying Enhances the Growth, Photosynthesis, and Resistance of Continuous *Pinellia ternata* and Promotes Its Yield and Quality. *Molecules* **2023**, *28*, 2053. [CrossRef] [PubMed]
32. Zhang, C.; Wu, Y.; Su, Y.; Xing, D.; Dai, Y.; Wu, Y.; Fang, L. A Plant's Electrical Parameters Indicate Its Physiological State: A Study of Intracellular Water Metabolism. *Plants* **2020**, *9*, 1256. [CrossRef] [PubMed]
33. Zhang, C.; Su, Y.; Wu, Y.; Li, H.; Zhou, Y.; Xing, D. Comparison on the Nutrient Plunder Capacity of *Orychophragmus violaceus* and *Brassica napus* L. Based on Electrophysiological Information. *Horticulturae* **2021**, *7*, 206. [CrossRef]
34. Zhang, C.; Wu, Y.; Su, Y.; Li, H.; Fang, L.; Xing, D. Plant's Electrophysiological Information Manifests the Composition and Nutrient Transport Characteristics of Membrane Proteins. *Plant Signal. Behav.* **2021**, *16*, 1918867. [CrossRef] [PubMed]
35. Wang, Q.; Li, H.; Lei, Y.; Su, Y.; Long, Y. Chitosan as an Adjuvant to Improve Isopyrazam Azoxystrobin against Leaf Spot Disease of Kiwifruit and Enhance Its Photosynthesis, Quality, and Amino Acids. *Agriculture* **2022**, *12*, 373. [CrossRef]
36. Zhang, C.; Long, Y.; Wang, Q.; Li, J.; Wu, X.; Li, M. The Effect of Preharvest 28.6% Chitosan Composite Film Sprays for Controlling the Soft Rot on Kiwifruit. *Hortic. Sci.* **2019**, *46*, 180–194. [CrossRef]
37. Zhang, C.; Li, Q.; Li, J.; Su, Y.; Wu, X. Chitosan as an Adjuvant to Enhance the Control Efficacy of Low-Dosage Pyraclostrobin against Powdery Mildew of Rosa roxburghii and Improve Its Photosynthesis, Yield, and Quality. *Biomolecules* **2022**, *12*, 1304. [CrossRef] [PubMed]
38. Wang, D.; Li, W.; Li, D.; Li, L.; Luo, Z. Effect of high carbon dioxide treatment on reactive oxygen species accumulation and antioxidant capacity in fresh-cut pear fruit during storage. *Sci. Hortic.* **2021**, *281*, 109925. [CrossRef]
39. Li, Y.; Wu, Y.; Liao, W.; Hu, L.; Dawuda, M.M.; Jin, X.; Yu, J. Nitric Oxide Is Involved in the Brassinolide-induced Adventitious Root Development in Cucumber. *BMC Plant Biol.* **2020**, *20*, 102. [CrossRef] [PubMed]
40. Li, J.; Quan, Y.; Wang, L.; Wang, S. Brassinosteroid Promotes Grape Berry Quality-Focus on Physicochemical Qualities and Their Coordination with Enzymatic and Molecular Processes: A Review. *Int. J. Mol. Sci.* **2023**, *24*, 445. [CrossRef] [PubMed]
41. Lin, F.; Cao, J.; Yuan, J.; Liang, Y.; Li, J. Integration of Light and Brassinosteroid Signaling during Seedling Establishment. *Int. J. Mol. Sci.* **2021**, *22*, 12971. [CrossRef] [PubMed]
42. Sukhov, V. Electrical signals as mechanism of photosynthesis regulation in plants. *Photosynth. Res.* **2016**, *130*, 373–387. [CrossRef] [PubMed]
43. Szechyńska-Hebda, M.; Lewandowska, M.; Karpiński, S. Electrical signaling, photosynthesis and systemic acquired acclimation. *Front. Physiol.* **2017**, *8*, 684. [CrossRef] [PubMed]
44. Xing, D.; Mao, R.; Li, Z.; Wu, Y.; Qin, X.; Fu, W. Leaf intracellular water transport rate based on physiological impedance: A possible role of leaf internal retained water in photosynthesis and growth of tomatoes. *Front. Plant Sci.* **2022**, *13*, 845628. [CrossRef] [PubMed]
45. Gallé, A.; Lautner, S.; Flexas, J.; Fromm, J. Environmental stimuli and physiological responses: The current view on electrical signaling. *Environ. Exp. Bot.* **2015**, *114*, 15–21. [CrossRef]
46. Sukhov, V.; Gaspirovich, V.; Mysyagin, S.; Vodeneev, V. High-temperature tolerance of photosynthesis can be linked to local electrical responses in leaves of Pea. *Funct. Front. Physiol.* **2017**, *8*, 763. [CrossRef] [PubMed]
47. Zhang, M.; Wu, Y.; Xing, D.; Zhao, K.; Yu, R. Rapid measurement of drought resistance in plants based on electrophysiological properties. *Trans. ASABE* **2015**, *58*, 1441–1446.
48. Xing, D.; Chen, X.; Wu, Y.; Zwiazek, J. Leaf physiological impedance and elasticity modulus in Orychophragmus violaceus seedlings subjected to repeated osmotic stress. *Sci. Hortic.* **2021**, *276*, 109763. [CrossRef]
49. Peres, A.L.; Soares, J.S.; Tavares, R.G.; Righetto, G.; Zullo, M.A.; Mandava, N.B.; Menossi, M. Brassinosteroids, the Sixth Class of Phytohormones: A Molecular View From the Discovery to Hormonal Interactions in Plant Development Stress Adaptation. *Int. J. Mol. Sci.* **2019**, *20*, 331. [CrossRef] [PubMed]
50. Junior, U.O.B.; Lima, M.D.R.; Alsahli, A.A.; Lobato, A.K.S. Unraveling the Roles of Brassinosteroids in Alleviating Drought stress in Young *Eucalyptus urophylla* Plants: Implications on Redox Homeostasis Photosynthetic Apparatus. *Physiol. Plant.* **2020**, *172*, 748–761. [CrossRef] [PubMed]

51. Zhang, C.; Zhang, Q.; Luo, M.; Wang, Q.; Wu, X. *Bacillus cereus* WL08 Immobilized on Tobacco Stem Charcoal Eliminates Butylated Hydroxytoluene in Soils and Alleviates the Continuous Cropping Obstacle of *Pinellia ternata*. *J. Hazard. Mater.* **2023**, *450*, 131091. [CrossRef] [PubMed]
52. Vlot, A.C.; Sales, J.H.; Lenk, M.; Bauer, K.; Brambilla, A.; Sommer, A.; Nayem, S. Systemic Propagation of Immunity in Plants. *New Phytol.* **2020**, *229*, 1234–1250. [CrossRef] [PubMed]
53. Rattan, A.; Kapoor, D.; Kapoor, N.; Bhardwaj, R.; Sharma, A. Brassinosteroids Regulate Functional Components of Antioxidative Defense System in Salt Stressed Maize Seedlings. *J. Plant Growth Regul.* **2020**, *39*, 1465–1475. [CrossRef]